

**RESEARCH ARTICLE**

**Morphological and Anatomical Study of Aerial organs of  
*Nepeta pannonica* L.**

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**ABSTRACT:**

The study of local medicinal plants has practical interest for the pharmaceutical industry in Kazakhstan, since the volume of domestic production satisfies not more than 10% of the total volume of consumed drugs. *Nepeta pannonica* L. is widespread herb used in folk medicine. The species can be used as a source of anti-inflammatory, antipyretic, antispasmodic and other agents. To introduce the species into the State Pharmacopoeia of Republic of Kazakhstan, its macroscopic and microscopic examination was carried out. Medicinal raw materials were studied using morphological and anatomical methods. Based on the results of macroscopic analysis, the following characteristic features were determined: the shape and color of the stem, the structure of the leaf (upper and lower sides, the type of venation), the shape of the inflorescence, the structure and color of the calyx and corolla. The following were identified as diagnostic features at the microscopic level: the shape and structure of the epidermal cells of the leaf and sepal, the degree and severity of pubescence, the shape and location of essential oil glands, the structure of the leaf and stem on a cross-section. The results obtained were included in the draft analytical regulatory document for medicinal vegetative raw materials of *Nepeta pannonica* L.

**KEYWORDS:** *Nepeta pannonica* L., Medicinal raw materials, Aerial organs, Macroscopic and microscopic analysis, Diagnostic signs.

**INTRODUCTION:**

The genus *Nepeta* L. belongs to the *Lamiaceae* Lindl. family (*Labiatae*) (<http://www.theplantlist.org/tpl1.1/record/kew-134873>), which plants have interest as sources of medicinal substances.

The genus *Nepeta* L. has more than 250 species, of which 16 species grow in Kazakhstan. Representatives of this genus have antioxidant, anticonvulsant, tonic, antipyretic, antitussive, sedative, anti-asthmatic, antispasmodic and anti-inflammatory properties<sup>1</sup>.

Pharmacological activity is explained by the content in the composition of different groups of biological compounds, such as steroids, organic acids, essential oils, tannins, flavonoids, vitamins and iridoids<sup>2</sup>.

Phenolpropanoids are also a part of various extracts of some *Nepeta* L. species. For example, chlorogenic, rosmarinic and chicoric acids were found to be the most common compounds. In addition, small amounts of kaempferol, *p*-coumaric acid, apigenin, luteolin and rhamnetin were quantitatively identified<sup>3</sup>.

*Nepeta pannonica* L. is a perennial herb 50-120 cm high. The plant is wide spread in Eurasia, including the Central Kazakhstan<sup>4</sup>.

According to scientists' data<sup>5</sup>, the following components of essential oils are present in large quantities in the aerial part of *N. pannonica*: 1,8-cineole and 4 $\alpha$ , 7 $\beta$ , 7 $\alpha$ -nepetolactone. *N. pannonica* is used in folk medicine<sup>6</sup>,

however, it has prospects for inclusion in the State Pharmacopoeia of the Republic of Kazakhstan (SP RK), which requires a pharmacognostic analysis.

The purpose of our study was to carry out analysis of the macro- and microscopic indicators of *N. pannonica* and identify the diagnostic signs of the plant.

**MATERIALS AND METHODOLOGY:**

The object of the study was the aerial parts (leaves, inflorescences and stems) of *N. pannonica*. This species was collected in the flowering phase, the place of collection - Spassky Sopki (the Karaganda region), 2nd decade of June 2021 (Fig.1). The species was identified by the staff of the Department of Botany of E.A. Buketov Karaganda University; the sample is placed in the herbarium fund of the Faculty of Biology and Geography.



Figure 1: *Nepeta pannonica* in the flowering phase (Spassky Sopki)

During the analysis of morphological parameters, the features of growth, appearance, surface structure, color of shoots, leaves, inflorescences and flowers were studied<sup>7-10</sup>. Samples of raw materials were examined through a Levenhuk DTX 30 Digital Microscope.

When performing anatomical studies, dry samples of aerial organs were soaked in hot water and softened in a mixture of glycerol-alcohol-distilled water in a ratio of 1:1:1<sup>11-15</sup>. Surface preparations and sections were prepared manually. Micrographs were taken on a BioMed scanning microscope using Altami Studio software at various magnifications. Photo processing was carried out in the Paint 10.1 program.

When describing morphology and anatomical structure, the principles set forth in the works of V.N. Vekhova, L.I. Lotova and recommendations of the Pharmacopoeia

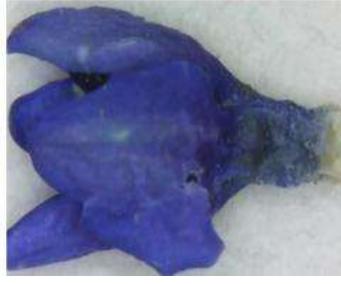
of the Republic of Kazakhstan were used<sup>16-21</sup>.

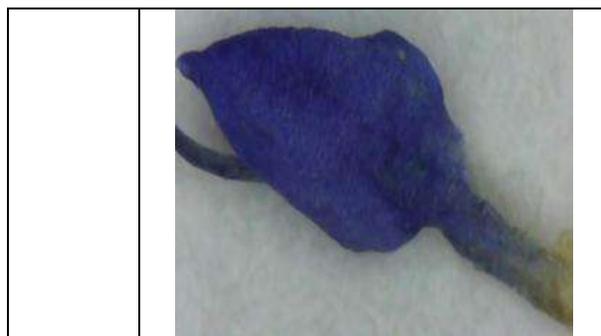
**RESULTS AND DISCUSSIONS:**

Morphological study. To determine the structural features of *N. pannonica*, we analyzed the indicators of the aerial organs and compiled the description presented in Table 1.

Table 1: Morphological indicators of aerial organs of *N. pannonica*

Indicators	Description
Shoots	Erect, branched and well frondose in the upper part, glabrous in the lower part. The color is light green in the lower and middle parts of the shoots. In the upper part with lilac or violet ribs. The leaves on the stem are sub-opposite.  
<b>Shoots pubescence</b>	<b>Shoots are glabrous along the entire length</b>
Leaves	The shape is narrowly elliptical, leaf tip is pointed, the base is cuneated, the edge is finely dentate, the venation is reticulate, the veins are clearly visible from the lower side.
Structure of the leaf's upper side	The upper side of the leaf is scabrous, with indistinct veins, the upper leaves are green, and the lower ones are purple-green.  
Structure of the leaf's lower side	The lower side is light green, the veins are well pronounced, the type of venation is reticulated. Numerous spot essential oil glandulars are evenly scattered over the entire surface.

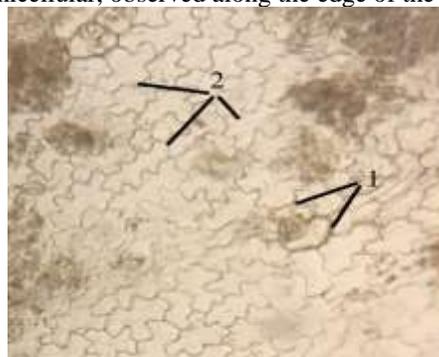
	
<p>Inflorescence shape</p>	<p>Apical inflorescences, narrowly paniculate, flowers per 3-6 are collected in whorls, length from 15 to 40(50)cm. Small awl-shaped bracts are placed on the inflorescence.</p> 
<p>Calyx</p>	<p>Calyx is shortly pubescent, 4-6mm long, radially symmetrical, teeth are short, subulate. The surface is finely ribbed. The color of the calyx outside is green; ribs and teeth of calyx are violet-colored. The teeth of calyx are almost naked, the base and middle part are densely pubescent with white hairs, mainly along the veins.</p> 
<p>Corolla</p>	<p>Corolla is twice as long as the calyx, 8-9 mm long, pale purple or pinkish</p> 



Thus, we have identified the following diagnostic features at the macroscopic level: shape and coloring of stem, leaf structure (upper and lower sides, venation type), inflorescence shape, structure and color of calyx and corolla.

**ANATOMICAL STUDY:**

The cells of the upper and lower epidermis of *N. pannonica* leaf are round or oval in shape with strongly sinuous walls (Fig. 2); above the veins of the leaf - rectangular and elongated (Fig. 3). The stomata are small, diacytic type, found on both sides of the lamina, but mainly on the lower side. Numerous large, rounded essential oil glands are scattered over the entire surface, lying at the level of the leaf epidermis. Trichomes are rare, unicellular, observed along the edge of the lamina.



1- Stomata, 2 - Main cells of the epidermis  
A



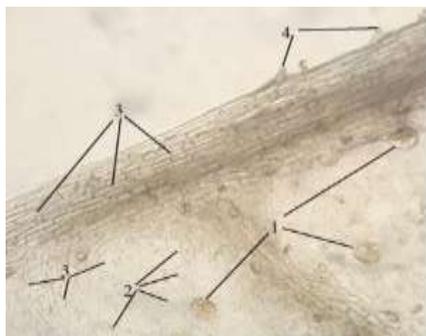
1 - Essential oil glands  
B



1 – Trichomes

C

**Figure 2:** Upper epidermis of *N. pannonica* leaf. Magnification 16x40: A - a fragment with the main cells of the epidermis and stomata, B - a fragment with essential oil glands, C - a fragment of the leaf edge with simple trichomes



1 – Essential oil glands, 2 – stomata, 3-main cells of the epidermis, 4 - trichomes

**Figure 3:** Lower epidermis of *N. pannonica* leaf. Magnification 16x40

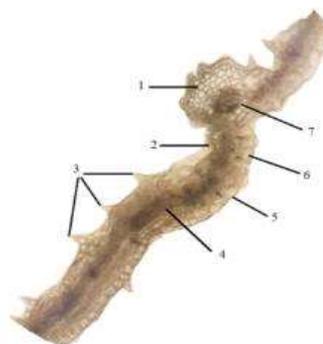
On a cross-section, the leaf of *N. pannonica* is flat, of the dorsoventral type (Fig. 4), with a pronounced division of the mesophyll into palisade and spongy tissues. On both sides, the leaf is surrounded by a 1-layer epidermis, the cells of which are oval in shape with thickened outer walls. Columnar mesophyll consists of 1-2 layers of cells.

The conducting bundle is of collateral-type, closed; consists of a xylem girder and a phloem girder. Above the main vein, there is a section of collenchymas (oriented to the lower side of the leaf). Rare simple trichomes and essential oil glands are clearly visible.



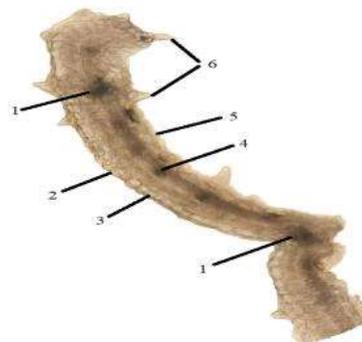
Essential oil gland

A



1-Collenchyma, 2-lower epidermis, 3-trichomes, 4- spongy mesophyll, 5- upper epidermis, 6- columnar mesophyll, 7- central conducting bundle

B



1 - Lateral conducting bundle, 2 - upper epidermis, 3 - columnar mesophyll, 4 - spongy mesophyll, 5 - lower epidermis, 6 – trichomes

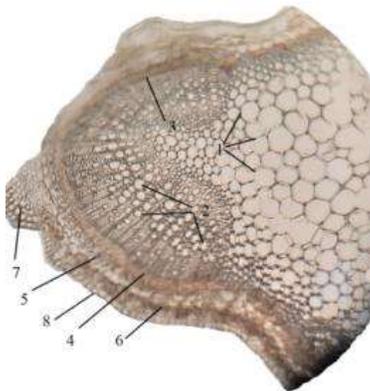
C

**Figure 4:** Cross-section of *N.pannonica* leaf. Magnification 16x10: A - a fragment with an essential oil gland, B - a fragment with a central vein, C - a lateral leaf fragment

Stem of *N. pannonica* is rectangular-lobed in cross-section (Fig. 5), of bundle type. A 1-layer epidermis with thickened cell membranes is located along the perimeter of the stem. Under the epidermis, there are areas of chlorenchyma, interrupted above the ribs by large areas of angular collenchymas. The crustal parenchyma lies between the conducting zone and the chlorenchyma. The conducting zone is limited to a single-layer endoderm. Bundles are collateral, of closed type. Bundles of 2 types are observed: in the corners - large broad-ovate bundles, between the corners - small and rounded. The xylem chains are well pronounced. In the center, the stem is filled with loose cells of the medullar parenchyma.



A



**B**  
1-Medullary parenchyma, 2- xylem, 3, 4- phloem, 5 - endoderm, 6 - chlorenchyma, 7- angular collenchymas, 8 – epidermis  
**Figure 5: Cross-section of *N. pannonica* stem. Magnification 16x10: A - general cross-section, B - a fragment of a cross-section**

On a surface preparation of *N. pannonica* sepal, the main cells are rectangular (Fig. 6) elongated with slightly sinuous walls. Simple and multi-cellular trichomes and large, rounded essential oil glands are clearly visible.



1- Maincells, 2- trichomes, 3 – essential oil glands  
**Figure 6: Superficial epidermis of *N. pannonica* sepal. Magnification 16x40:**

The results of the study of the superficial epidermis of the corolla of *N. pannonica* flower, presented in Figure 7, indicate that the main cells are rectangular in shape with thin walls. Rare simple trichomes and rounded essential oil glands are clearly visible.



1- Trichomes, 2 – essential oil glands  
**Figure 7: Superficial epidermis of *N. pannonica* corolla. Magnification 16x40**

The obtained microscopic signs of *N. pannonica* raw materials were summarized in Table 2.

**Table 2: Microscopic indicators of *N. pannonica* aerial organs**

Indicators	Description
Upper epidermis of the leaf	Rounded or oval with highly sinuous walls
Lower epidermis of the leaf	Rounded or oval in shape with highly sinuous walls; above the veins of the leaf - rectangular and elongated
Stomata type, localization	Small, diacytic type
Essential oil glands of leaf epidermis	Numerous large, rounded essential oil glands are scattered over the entire surface, lying at the level of the leaf epidermis
Trichomes	Trichomes are rare, unicellular, observed along the edge of the lamina
Leaf type in cross-section	The leaf is flat, of dorsoventral type, but with a pronounced division of the mesophyll into palisade and spongy tissues
Calyx	Epidermal cells are elongated, with slightly sinuous walls; rare, small multicellular trichomes and large essential oil glands are scattered over the surface
Corolla	The cells are rectangular, with thin walls; on the surface there are rare simple unicellular trichomes and rounded essential oil glands. Multicellular hairs are placed along the edge of the corolla
Stem shape in cross-section	Rectangular laciniate, of beam type
Type of conducting system	Of collateral type, closed, beams of two types - large, small

Analysis of the data obtained allows us to determine the following microscopic diagnostic features: the shape and structure of the epidermal cells of the leaf and sepal, the degree and severity of pubescence, the shape and location of essential oil glands, the structure of the leaf and stem on a cross-section.

### CONCLUSION:

Based on the results of the studies, macro- and microscopic features of *N. pannonica* raw materials were determined:

**For the leaf:** Macroscopic features: the shape of the lamina, tops, bases and edges, more pronounced veins on the lower side of the leaf. Microscopic features: shape of epidermal cells, tortuosity of walls, stomata of diacytic type, large essential oil glands and rare trichomes.

**For the stem:** The form of stem growth, branching is mainly in the upper part, color, sub-opposite arrangement of leaves. Microscopic features: the shape of the stem on the cross-section, the structure of the bundles of 2 types.

**For the inflorescence:** Macroscopic features: type of inflorescence, peculiarities of the arrangement of flowers and bracts in the inflorescence.

**For the calyx:** Macroscopic features: the shape of the calyx and teeth of the calyx, the type of pubescence - mainly along the ribs, color, the presence of small ribs on the surface; the surface is finely ribbed. The color of the calyx outside is green; ribs and teeth of the calyx are violet-colored. Microscopic feature: cells of the epidermis are elongated, with slightly sinuous walls, on the surface the shape of epidermal cells, the presence of small multi-cellular trichomes and large essential oil glands.

**For the corolla:** Macroscopic features: the shape and size of the corolla, the color is pale purple or pinkish. Microscopic features: the shape of epidermal cells, the presence of simple unicellular trichomes on the surface and multi-cellular trichomes along the edge, large essential oil glands.

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#### CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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